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CHILLING REQUIREMENTS FOR OPTIMAL GROWTH OF ROCKY MOUNTAIN DOUGLAS-FIR SEEDLINGS

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ABSTRACT

*One-year-old Douglas-fir (*Pseudotsuga menziesii *var.* glauca*) seedlings from three contrasting interior provenances were chilled for periods ranging from 2 to 20 weeks. Chilling treatments were: a constant 4°C, a fluctuating 4°C night and 10°C day, and the outdoor environment. Analyses of six variables related to growth and phenology suggest that the chilling requirements of Rocky Mountain Douglas-fir seedlings were best satisfied by 17 weeks of constant 4°C.*

KEYWORDS: Douglas-fir, chilling requirement, chilling period

In the fall, woody plants of temperate climates undergo physiological changes that provide resistance to cold during the winter. The internal changes prevent growth until the plant has been exposed to cool temperatures. The range and duration of exposure to cool temperatures required before growth can resume is the "chilling requirement." The chilling requirements of numerous conifers have already been assessed: *Pseudotsuga menziesii* var. *menziesii* (Wommack 1964; Lavender and Hermann 1970; Campbell and Sugano 1975; van den Driessche 1975), *Pinus strobus* (Berry 1965), *Pinus monticola* (Steinhoff and Hoff 1972), *Picea abies* (Worrall and Mergen 1967; Dormling and others 1968), and seven species of *Picea* (Nienstaedt 1966, 1967).

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In general, 3°-6°C is the most effective chilling temperature for most species. However, species vary greatly in the duration of required chilling. For example, 4 weeks is required for *Picea abies* (Dormling and others 1968) while 16 weeks is required for *Pinus monticola* (Steinhoff and Hoff 1972).

In the Northern Rocky Mountains, Douglas-fir (*P. menziesii* var. *glauca*) seedlings are being container-grown in greenhouses, shadehouses, and growth chambers for both reforestation and research. Although the coastal Douglas-fir (*P. menziesii* var. *menziesii*) requires a chilling period of 8-12 weeks at 3°-6°C (Wommack 1964; Lavender and Hermann 1970; Campbell and Sugano 1975; van den Driessche 1975), information on the chilling requirements of the Rocky Mountain variety is lacking. Yet, in order to obtain optimal growth from container-grown seedlings, the chilling requirement must be known.

The primary objective of the study reported here was to determine the chilling requirement of Rocky Mountain Douglas-fir seedlings in both a controlled environment and the outdoor environment. A secondary objective was to detect possible differences in chilling requirements among provenances. For this study, the chilling requirement was considered satisfied when additional chilling resulted in no increase in growth.

MATERIAL AND METHODS

Two separate experiments were established. Both used seed from three ecologically and geographically contrasting provenances (table 1). For each provenance, 60-75 seed were sown in each of eighteen 8-inch (20-cm) pots. After the seed had germinated in the greenhouse, all seedlings (approximately 50 per pot) were moved to a shadehouse to complete their first year of growth. After bud set and following the first hard frost (below -3°C), which occurred October 17, studies of the chilling requirements were begun.

Experiment I.--To determine the amount of chilling required for seedlings in the outdoor environment, six pots of seedlings from each provenance were placed under a bench in the lathhouse and were mulched with sawdust. One pot of seedlings from each provenance was moved into the greenhouse after 2, 8, 11, 14, 17, and 20 weeks. A continuous temperature record was obtained by placing a hygrothermograph with the seedlings (fig. 1).

Experiment II.--The second experiment was designed to assess chilling period requirements of seedlings under two temperature regimes:

Treatment 1. 4°C constant with moderate light (300 ft-c; 3,230 lux) for 8 hours per day.

Treatment 2. 10°C, 8-hour day with moderate light (300 ft-c; 3,230 lux) and 4°C, 16-hour night.

For each treatment, six pots of seedlings from each provenance were placed in a "holding" chamber set for a 16°C 10-hour day and a 10°C 14-hour night. These settings were used to keep the seedlings at the first stage or dormant-quiescent stage of acclimation (Weiser 1970) without providing chilling. For each treatment, one pot of seedlings from each provenance was taken from the "holding" chamber and placed into a chilling chamber at intervals that provided 2, 8, 11, 14, 17, and 20 weeks of chilling at the end of 20 weeks. Although seedlings spent a variable amount of time in the "holding" chamber, the end of the chilling treatment occurred on the same date for all seedlings. Consequently, effects of the treatments on growth and development of the seedlings could be observed under uniform conditions.

Table 1.--*Provenances of Rocky Mountain Douglas-fir represented in chilling study*

Provenance No.	Latitude	Longitude	Elevation	National Forest	Habitat type
			Meters		
4	45°50'	115°55'	1,060	Nezperce	*Abies grandis/ Pachistima myrsinites*
18	48°35'	118°35'	1,430	Colville	*Abies lasiocarpa/ Pachistima myrsinites*
22	48°21'	117°15'	885	Colville	*Thuja plicata/ Pachistima myrsinites*

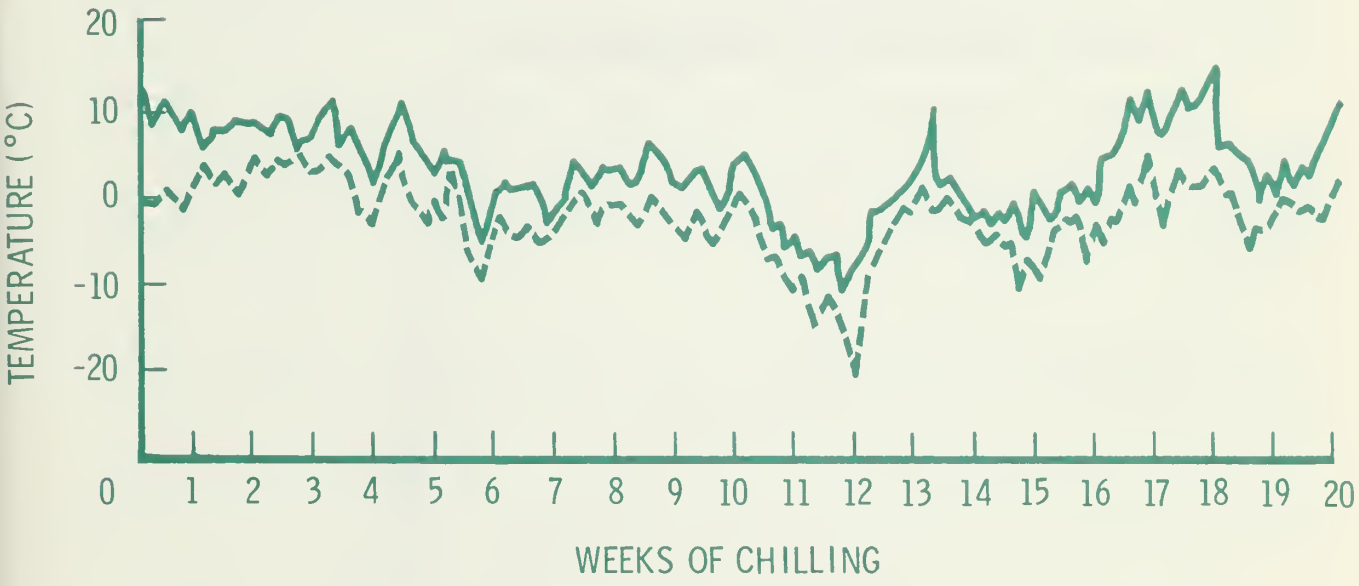

Figure 1.--Daily maximum and minimum temperatures recorded by hygrothermograph during outdoor chilling of seedlings in Experiment I.

In both experiments, chilled seedlings were moved to a greenhouse maintained at 21°-24°C, with a 14-hour photoperiod of sunlight extended with a combination of fluorescent and incandescent lighting. All seedlings were checked twice weekly and the following data taken on individual seedlings: number of days to terminal bud burst, number of days to formation of the first terminal bud, total height, and epicotyl elongation.

Analyses of variance were used to assess the chilling requirement of each provenance. These analyses were based on the means of all seedlings within a pot for: total height, amount of elongation, days to bud burst and bud set, duration of growth, and deviation from predicted growth. The last variable was the deviation from regression of total height on first-year height. As such, it represents rate of growth from a constant initial height.

RESULTS

Although no statistical comparisons could be made between the two experiments, differences were obvious. Only 75 percent of the seedlings in Experiment I burst bud, whereas 90 percent of the seedlings in Experiment 11 burst bud. Secondly, seedlings in Experiment 1 averaged 0.24 cm shorter, 2-1/2 days later in bud burst, 9 days later in bud set, and 6-1/2 days longer in duration of growth. Generally, the outdoor environment provided less effective conditions for satisfying chilling requirements than the chilling chambers.

Experiment I.--Duration of chilling period had a pronounced effect on the percentage of seedlings which burst terminal bud (fig. 2). Analysis of variance showed that chilling periods had significant effects on only bud burst, bud set, and deviation from the regression of final height on initial height (table 2A). Effects of chilling period are evident in the mean values associated with increasing duration of chilling period (table 2B). The longer the chilling period, the earlier bud burst and bud set and the greater the growth from a constant initial height.

Main effects of provenances were significant for date of bud burst, date of bud set, total height, and shoot elongation. On the average, seedlings from provenance 18 burst bud 3 days later and set bud 4 days later than seedlings from the other two provenances. Even though provenances differed in total height and elongation, it is noteworthy that these differences were entirely the result of first-year growth. When total height was adjusted for first-year height, no differences could be detected among provenances.

Experiment II.--Chilling temperature significantly affected bud burst and duration of growth (table 3A). Seedlings chilled at 4°C constant temperature burst bud about 2 days earlier and grew about 1-1/2 days longer than those chilled at 4°-10°C fluctuating temperature. Yet in general, few differences were obvious between treatments.

Increases in duration of chilling period up to 17 weeks resulted in earlier bud burst and progressive increases in growth (table 3; fig. 3). After 17 weeks of chilling, little effect of an increased chilling period was evident.

Effects of provenances were significant for several variables. These effects paralleled those detailed for Experiment 1. In general, interactions involving provenances were insignificant. This suggests that chilling requirements are similar for all provenances tested.

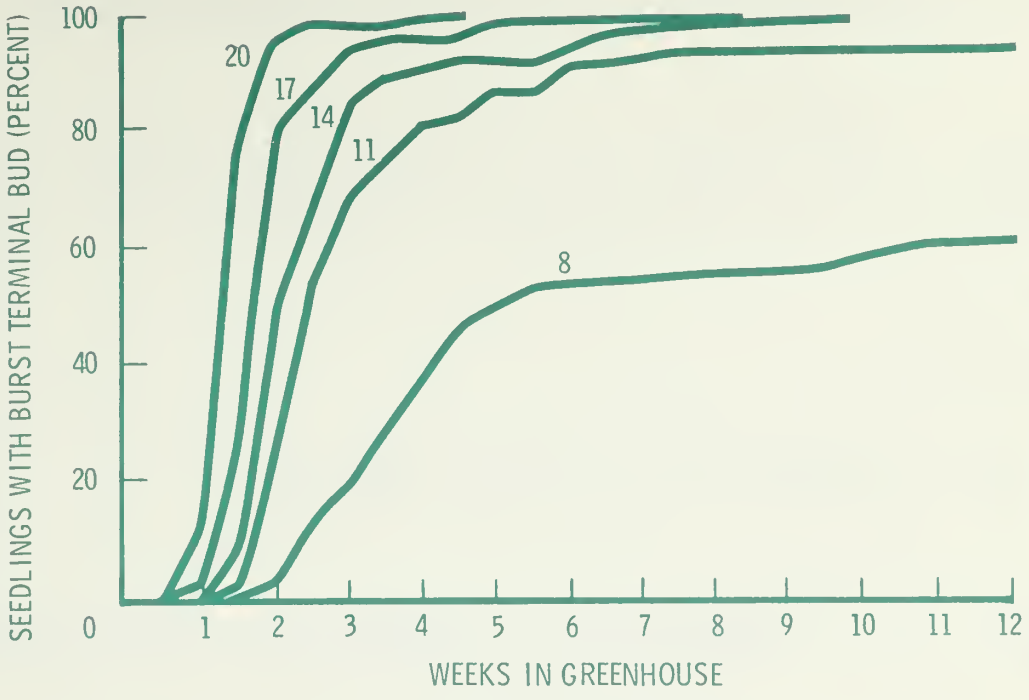

Figure 2.--Experiment I (lathhouse) - relationship between cumulative percentage of seedlings that burst terminal bud and weeks in greenhouse for various chilling periods.

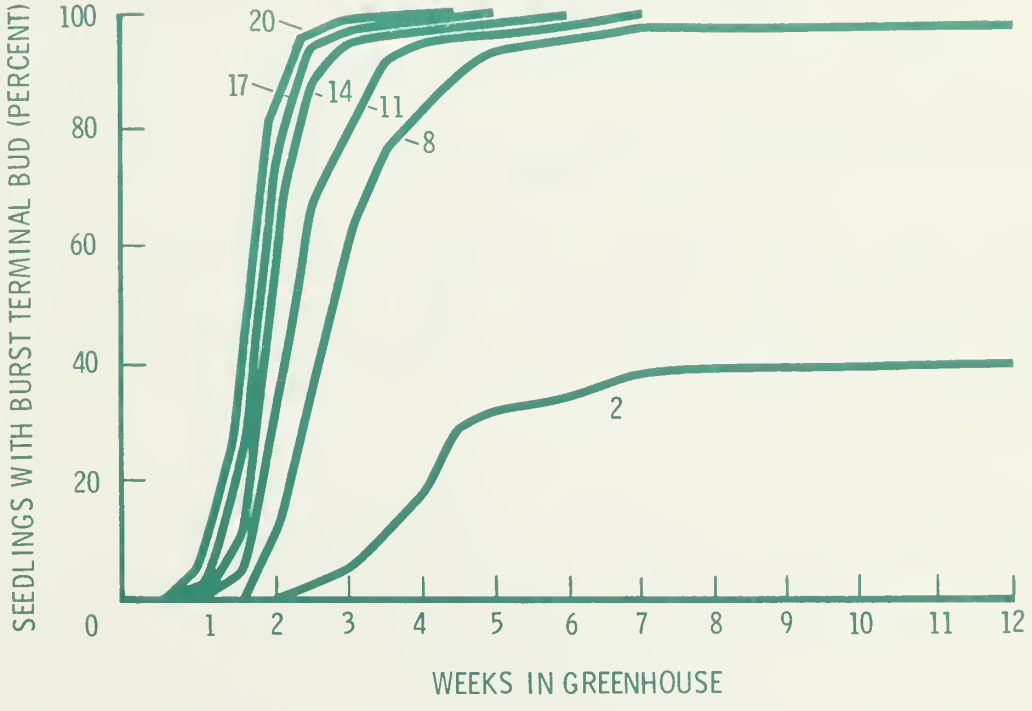

Figure 3.--Experiment II (growth chambers) - relationship between cumulative percentage of seedlings that burst terminal bud and weeks in greenhouse for various chilling periods.

Table 2.--*Results of Experiment I. Part A: Analysis of variance. Part B: Mean values of six variables according to chilling period*

A.

Source of variation	Degrees of freedom	Total height	Elongation	Deviation from predicted growth	Bud burst	Bud set	Duration of growth
Provenance	2	**	**	NS	**	*	NS
Chilling period	4	NS	NS	**	**	**	NS
Provenance X Chilling period	8						

* Denotes significance at the 0.95 level of probability.
** Denotes significance at the 0.99 level of probability.

B.

Chilling period	Total height	Elongation	Deviation from predicted growth	Bud burst	Bud set	Duration of growth
Weeks	*Centimeters*			*Days*		
2	Only one seedling burst bud					
8	7.76	2.84	-0.81	30.3	52.7	22.4
11	8.66	3.69	.02	21.6	47.6	26.0
14	8.27	3.60	.12	18.8	41.4	22.6
17	8.35	3.60	.16	14.8	39.7	24.9
20	8.46	3.92	.51	10.9	33.1	22.2

DISCUSSION

Results of this study describe the chilling requirements of seedlings from three provenances. Data on growth and phenology were obtained from the first period of shoot elongation during the second growing season. However, seedlings usually flush and set terminal bud two or three times during the second growing season under optimal conditions. But, because the first flush of growth is from a preformed bud and subsequent flushes are from buds developed during the current growing season, chilling directly affects only the first flush.

Results of Experiment I showed that an increase in chilling period was accompanied by progressive changes in three variables: elongation, date of bud burst, and growth. This suggests that for the year tested, 20 weeks of chilling in the natural environment may have been insufficient for realizing the maximum growth potential of the seedlings tested. However, a comparison of mean response of seedlings to 20 weeks of chilling in Experiment I with those of a comparable chilling period in Experiment II suggests that 20 weeks of chilling in the natural environment was very close to being optimal.

A 20-week chilling period represents the requirement of seedlings for only one winter at Moscow, Idaho. The controlled environment experiments had warmer average temperatures and the optimum chilling period was 17 weeks. Therefore, it seems that 20 weeks of chilling should be considered maximum for most Rocky Mountain environments.

Table 3.--*Results of Experiment II. Part A: Analysis of variance for Experiment II. Part B: Mean values of six variables according to chilling periods*

A.

Source of variation	Degrees of freedom	Total height	Elongation	Deviation from predicted growth	Bud burst	Bud set	Duration of growth
Treatment	1	NS	NS	NS	**	NS	*
Provenance	2	**	**	NS	**	**	*
Chilling period	5	NS	**	**	**	**	**
Treatment X Provenance	2	NS	NS	*	NS	NS	NS
Treatment X Chilling period	5	NS	NS	NS	NS	NS	NS
Provenance X chilling period	10	NS	NS	NS	*	NS	NS
Treatment X Provenance X Chilling period	10						

* Denotes significance at the 0.95 level of probability.
** Denotes significance at the 0.99 level of probability.

B.

Chilling period	Total height	Elongation	Deviation from predicted growth	Bud burst	Bud set	Duration of growth
Weeks	*Centimeters*			*Days*		
2	8.61	2.94	-1.27	31.7	43.2	11.5
8	8.97	3.71	-0.25	22.1	38.6	16.5
11	8.71	3.83	.11	18.3	36.1	17.8
14	9.06	4.00	.17	15.5	32.1	16.6
17	9.26	4.43	.73	14.2	32.6	18.4
20	9.16	4.25	.51	13.6	31.0	17.4

In Experiment II, bud burst occurred progressively earlier with an increase in duration of chilling period. However, variables related to growth potential culminated at about 17 weeks of chilling. As suggested by Campbell and Sugano (1970), bud burst may not culminate with increased chilling, but the range of temperatures at which growth begins may increase to the point that growth begins at chilling temperatures. Consequently, optimal growth should be the main criterion by which effectiveness of chilling treatments is measured. Therefore, the results of Experiment II suggest that 17 weeks of chilling at 4°C constant temperature best satisfied the chilling requirement of the Rocky Mountain Douglas-fir seedlings tested.

These results are in general agreement with the findings of van den Driessche (1975). He concluded that 15 weeks of chilling at 4.4°C fulfilled the chilling requirements for seedlings from a single interior British Columbia provenance of Douglas-fir.

Results of analyses of variance suggested that the provenances tested required a similar chilling duration. Although seedlings from provenance 18 performed very differently from those from the other two provenances, analysis failed to detect interactions involving provenances. However, these analyses included data from only those seedlings that had burst bud. When provenances are compared according to the percentage of seedlings that had burst bud in all treatments of both experiments, provenances differ significantly (0.95 level of probability). Altogether, 87 percent of the seedlings from provenances 4 and 22 burst bud, but only 80 percent of those from provenance 18 burst bud. Moreover, these differences were consistent in both experiments. As a consequence, it seems that the major difference between the provenances tested is in the number of seedlings which had their chilling requirements satisfied by a given chilling treatment rather than in performance subsequent to bud burst.

CONCLUSIONS

From the findings of this study, the following recommendations can be made concerning chilling requirement of Rocky Mountain Douglas-fir seedlings: First, if chilling is done in an outdoor environment similar to Moscow's, 20 weeks of chilling are recommended after the first hard frost. Second, at least 17 weeks of chilling at 4°C is recommended in controlled environments.

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